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Letters

T Cell–Based Assay of Pericardial Fluid Mononuclear Cells for the Diagnosis of Tuberculous Pericardial Effusion

Diagnosing tuberculous pericardial effusion (TPE) remains a challenge despite continuing improvements in microbiological and molecular techniques. Recently, an enzyme-linked immunosorbent spot (ELISPOT) assay was developed to count the numbers of antimycobacterial effector T cells that produce interferon (IFN)-gamma against *Mycobacterium tuberculosis*-specific antigens encoded by genes located in region of difference 1. However, the clinical feasibility and usefulness of this method for diagnosing TPE have not been demonstrated.

We prospectively enrolled 35 consecutive patients with a moderate to large amount of newly diagnosed pericardial effusion who were hospitalized at Asan Medical Center between July 1, 2008 and February 28, 2011. The hospital's institutional review board approved the study protocol, and patients' informed consents were obtained. All patients underwent pericardiocentesis or a pericardial window operation. Microbiological and pathological specimens were processed for diagnosis of TPE. ELISPOT assays (T-SPOT.TB, Oxford Immunotec, Abingdon, United Kingdom) with *M. tuberculosis*-specific antigens (ESAT-6 and CFP-10) were performed as previously described (1). Mononuclear cells from the peripheral blood and pericardial fluid samples were used. A negative control (NC) is a duplicate well containing no antigen, and a positive control is a well containing phytohemagglutinin. The resulting spots were counted using an automated microscope (ELiSpot 04 HR, Autoimmune Diagnostika GmbH, Strassberg, Germany). When an extremely high number of sensitized T lymphocytes was counted as 1 by automatic spot counting, the number was corrected to 2000. The differences between the numbers of spots in the specific antigen-containing wells and NC wells were used as continuous diagnostic variables to obviate the effect of background debris in the pericardial effusion.

Adenosine deaminase (ADA) activity in pericardial effusion was also measured. Decisions regarding antituberculosis therapy were made by the attending physicians on the basis of the laboratory findings and clinical features of each patient, except for ELISPOT assays results. The study investigators, without knowledge the ELISPOT assay results, categorized patients into 2 groups, TPE and non-TPE (NTPE), using previously reported diagnostic criteria (2).

Of 35 patients, 12 and 23 patients were diagnosed with TPE and NTPE, respectively. In the NTPE group, 11 and 12 patients were diagnosed with malignant and idiopathic pericardial effusions, respectively. The receiver-operating characteristic (ROC) curve analysis showed that the areas under the curves of ELISPOT assays using peripheral blood were 0.737 for Δ(ESAT-6 - NC) and 0.792 for Δ(CFP-10 - NC), whereas those of ELISPOT assays using pericardial fluid were 0.895 for Δ(ESAT-6 - NC) and 0.895 for Δ(CFP-10 - NC). When pericardial fluid was used, the optimal cutoff values for the diagnosis of TPE were 39 for Δ(ESAT-6 - NC) and 92 for Δ(CFP-10 - NC). When these cutoff levels were used, the sensitivity and specificity were 92% and 87% by Δ(ESAT-6 - NC) and 75% and 100% by Δ(CFP-10 - NC), respectively. The acid-fast bacilli stain and cultures for *M. tuberculosis* were performed on all 35 patients. The results of the acid-fast bacilli stain on pericardial effusion were negative in all 35 patients, and cultures for *M. tuberculosis* were positive in 3 patients. Polymerase chain reaction for *M. tuberculosis* was performed in 31 (89%) patients, and only 1 patient showed a positive result. Of the 15 (43%) patients who underwent pericardial biopsy, granuloma was found in the pericardial tissue of 1 patient. The ADA level in pericardial fluid was measured in 30 patients (86%). The area under the ROC curve of ADA levels was 0.931. The optimal cutoff value was 42 U/l, and the sensitivity and specificity by this cutoff value were 92% and 89%, respectively. The area under the ROC curve was 0.981 when a combination of ADA and ELISPOT assay results of pericardial fluid was used for the diagnosis of TPE (**Figure 1A**). Compared with ADA level, net reclassification index of a combination of ADA and ELISPOT results was 0.4167 (95% confidence interval: 0.1075 to 0.7259). When positive results in ≥2 of 3 variables (ADA level, Δ

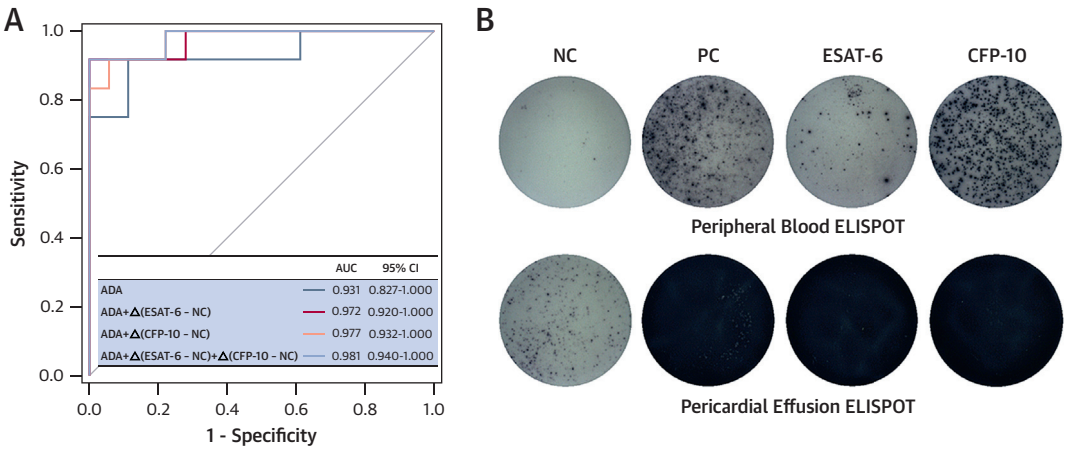

FIGURE 1 Diagnosis of Tuberculous Pericardial Effusion by the ELISPOT Assay

(A) Receiver-operating characteristic curve analysis for the diagnosis of tuberculous pericardial effusion by adenosine deaminase (ADA) level and results from the enzyme-linked immunosorbent spot (ELISPOT) assay using pericardial effusion. **(B)** An example of ELISPOT using peripheral blood and pericardial effusion in patients with definitive tuberculous pericardial effusion. **Solid black plates** were entirely packed with numerous spots, suggestive of an extremely high density of sensitized lymphocytes. AUC = area under the curve; CI = confidence interval; NC = negative control; PC = positive control.

[ESAT-6 - NC] and Δ[CFP-10 - NC] on pericardial effusion), as judged using the cutoff values determined in this study, were used as a diagnostic criterion for TPE, sensitivity, specificity, and positive and negative predictive values were 92%, 100%, 100%, and 95%, respectively. In 4 patients classified as *definitive* TPE, defined as detection of acid-fast bacilli in a stained smear or culture of pericardial fluid or detection of tubercle bacilli or caseating granuloma on histological examination of the pericardium (3), extremely strong positive reactions in the *M. tuberculosis*-specific antigen-containing wells and positive controls were found when pericardial fluid was analyzed using the ELISPOT assay (**Figure 1B**).

The detection rate of tubercle bacilli on direct smear examination of pericardial effusion is reportedly low, ranging from 0% to 42%. The conventional culture of tubercle bacilli from pericardial fluid has a detection rate of 53% (4). *M. tuberculosis* detection by polymerase chain reaction has a low yield, ranging from 50% to 81%. Therefore, it is not a suitable diagnostic tool (5). Measuring ADA activity in pericardial effusion is a useful method for diagnosing TPE with a high sensitivity of 90%, but its diagnostic power is limited by its relatively low specificity of ~74% (2). Our results indicate that the ELISPOT assay using pericardial fluid is a useful method for the diagnosis of TPE and that it enhances specificity when used in combination with the assessment of ADA activity in pericardial effusion.

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Circadian Variations of Ventricular Arrhythmias and Sleep-Disordered Breathing in HF Patients

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We read with interest the study by Patton et al. (1) on the unexpected absence of typical circadian variation of ventricular arrhythmias observed in the SCD-HeFT (Sudden Cardiac Death in Heart Failure Trial). Sleep-disordered breathing (SDB), broadly categorized into obstructive and central sleep apnea, has been associated with increased cardiovascular morbidity and mortality. Cardiac arrhythmias are responsible for some of the higher cardiovascular morbidity and mortality rates observed in patients with SDB. The association between atrial fibrillation and SDB is well established, although the association between SDB and life-threatening ventricular arrhythmias also seems plausible. Obstructive sleep apnea predicts sudden cardiac death independently of other well-established risk factors (2), and, unlike the general population, patients with SDB have a higher incidence of sudden cardiac death during sleep (3). Studies consistently report an SDB prevalence of ≥50% in the chronic heart failure population. The prevalence of SDB in patients with an implantable cardioverter-defibrillator (ICD) ranges between 57.8% and 66.3% (4,5). In a cohort of 472 ICD patients with heart failure receiving cardiac resynchronization therapy, a significant risk enhancement of ventricular arrhythmias and appropriate ICD therapies owing to both central and obstructive sleep apnea was found (5). Importantly, for heart failure patients with a primary inappropriate ICD therapies (4). Patton et al. (1) observed an increase in the onset of ventricular arrhythmias during sleep in patients with an ICD and SDB. Data on SDB for patients enrolled in the SCD-HeFT were not reported. Thus, it is our opinion that the observed deviation in circadian variation of ventricular arrhythmias reported by Patton et al. (1) may be influenced, at least in part by the presence of SDB, a very prevalent condition among heart failure patients with an ICD.

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REPLY: Circadian Variations of Ventricular Arrhythmias and Sleep-Disordered Breathing in HF Patients

We appreciate the interest of Dr. Arias and colleagues in our study of circadian and septadian patterns of implantable cardioverter-defibrillator therapy in the SCD-HeFT (Sudden Cardiac Death in Heart Failure Trial) population (1). In their Letter, they relevantly highlight the importance of sleep-disordered breathing as a trigger of ventricular arrhythmias and implantable cardioverter-defibrillator therapies. Both central and obstructive sleep apnea exert strong effects on the autonomic nervous system and are known to be proarrhythmic (2).

We agree with Dr. Arias and colleagues that sleep-disordered breathing is an important and increasingly recognized trigger of arrhythmias (3). Unfortunately, we do not have information on the presence